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MEDICINAL PLANTS RHIZOSPHERE – A BOON FOR INHABITING ANTIBIOTIC PRODUCING MICROORGANISMS

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ABSTRACT

To explore the production of antibiotics from soil microbes residing in the rhizosphere of medicinal plants, a study was carried out at Himachal Pradesh, a hilly state situated in northern Himalayas. Microbes were isolated from the rhizospheric region of three medicinal plants viz., Aloe vera, Mentha and Salvia. Obtained isolates were compared with each other and the isolate having high cfu (colony forming unit) in a particular rhizosphere was selected for further study. For antibiotic sensitivity test (AST) the obtained isolate was clinically screened against three pathogenic microorganisms which were obtained from IGMC (Indira Gandhi Medical College, Shimla) through regular isolations at Cary-Blair transport medium. These four isolates were identified through morphological and biochemical studies and then were fed into ABIS (Advanced Bacterial Identification Software) which identified these as Bacillus from Aloe vera rhizosphere and 3 other pathogenics as Staphylococcus, Pseudomonas and E.coli. Results obtained during AST was expressed in terms of the diameter of the inhibition zone caused by Bacillus against clinical isolates. Highest zone diameter of 26mm was observed in Pseudomonas followed by Staphylococcus and E.coli.

KEYWORDS: Rhizosphere, Aloe vera, Mentha and Salvia

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Original Article

INTRODUCTION

In day to day language the word “antibiotic” means “against life. However, in science we use the word to describe a set of chemicals that inhibit or kill bacterial pathogens (Mondena et al., 1996). The British scientist Alexander Fleming is credited with being the first to notice that one bacteria could inhibit the growth of other one by releasing some antibiotics. The plants having medicinal properties and have potential use as antimicrobial, antipyretic, anti-cancerous agents etc., are termed as medicinal plants (Parmar and M Rawat, 2012; Choudhary et al., 2015). Many medicinal plants viz., *Aloe vera*, *Mentha*, *Salvia* leaf bears many medicinal properties like reduction in overproduction of perspirations and also used in treatment for depression, memory loss, and Alzheimer’s diseases (Singhal et al., 2012). Literature cited that many microbiologists isolated different types of microorganisms from rhizosphere of these medicinal plants (Nirmala et al., 2016; Tamilarasi et al., 2008). Members of the *Bacillus* genus are generally found in soil and represent a wide range of physiological abilities, allowing the organism to grow in every environment and compete desirably with other organisms within the environment due to its capability to form extremely resistant spores and produce metabolites that have antagonistic effects on other microorganisms (Tamilarasi et al., 2008). Also species from the genus *Bacillus* are considered as safe microorganisms and they possess remarkable abilities to synthesize many substances that have

been successfully used in clinical purposes (Olmos, and Paniagua-Michel, 2014). The secondary metabolites produced by several species and strains of the genus *Bacillus* have been found to show antibacterial activity against different pathogenic microorganism. The genus *Bacilli* has been found is produce high preparation of antimicrobial compound including bacitracin, polymyxin, gramicidin, tyrocidine, subtilin, bacilysin and also have bactericidal effects on both gram positive and gram negative bacteria, therefore used as biocontrol agent (Dhanasekara et al., 2005). The paper therefore highlights the isolation of bacteria from rhizospheric soils of medicinal plants and the isolated one was affectively screened against pathogenic bacterial isolates.

MATERIAL AND METHODS

Collection of Soil Samples

Our sampling site Mandi, situated in India within Himachal Pradesh province in between 31^{o}72'North latitude 76^{o}92' East longitude with an elevation of 1044 metres (3,425 feet). With the help of sterile spatula soil samples were collected at a depth of 5cm from rhizospheric soils of *Aloe vera*, *Salvia* and *Mentha* in polythene bags. Samples were dried separately at 45°for 1 hour in a hot air oven, cooled to room temperature and further isolations were carried out.

Isolation and Maintenance of Microbial Cultures

One gm of each soil sample, suspended in 10ml of sterile saline water (0.86% NaCl) and vortexed. A series of dilutions from 10^{-1} to 10^{-7} were made, these dilutions were used in the spread plate method. The resulting serial dilutions of soil (0.1 ml) were pipette on nutrient agar (Himedia, India) media in triplicates. The plates were incubated at 37°C for 24 h and observed intermittently. Maintenance of microbial cultures were done on nutrient agar slants and then preserved at 4°C.

Isolation of Clinical Isolates

Bacterial samples were also procured from Department of Microbiology, IGMC (Indira Gandhi Medical College, Shimla) Himachal Pradesh. Samples were isolated on Cary-Blair transport medium. The isolated pathogenic microorganisms were screened for antimicrobial sensitivity test.

Identification of Bacterial Isolates

For physiological culture identification, isolates were grouped on the basis of phenotypic characteristics such as colony colour, appearance, elevation, and margins on nutrient agar. Other characters such as endospore staining, capsule staining and Gram reaction (for cocci and bacilli detection) and cell arrangement were also recorded. The photos were slightly refined in sharpness and color tone with CorelDRAW X4. Routine biochemical tests such as indole; methyl red; Voges–Proskauer; citrate; the presence of oxidase and catalase; sugar utilization were assessed for each bacteria. Clustering of biochemical analysis was done with SXL software. The results obtained through morphological and biochemical tests were fed into ABIS (Advanced Bacterial Identification Software (http://www.tgw1916.net/software.html) to identify the isolates at genus level.

Antibiotic Sensitivity Test

Bacterial growth scrapped aseptically from 48 hours old agar slants. Inoculated to 50ml sterilized basal medium in 250ml conical flask and shaken on rotatory shaker at 150rpm for 48 hours at 37°C. At the end of fermentation period, fermented material was centrifuged. The antibiotic present in the fermented material was determined by both Agar well

diffusion method and Disc diffusion method on the Muller Hinton agar medium.

Screening of Isolate for Antimicrobial Activity

Agar Well Diffusion Method

The aim of this experiment was to compare the antimicrobial effectiveness of the selected rhizobacterial isolate against pathogenic isolates. Different Muller Hinton agar Media plates of suitable media were prepared and inoculated with 1ml of respective pathogenic organisms by spread plate method. Wells measuring 3 mm in diameter were cut out under aseptic conditions using a stainless steel borer (Magaldi et al., 2004; Valgas et al., 2007). Each well was filled with 25 ul extract of *Bacillus* and incubate at 37°C.A clear zone of inhibition, which is more than 12 mm, was considered to be sensitive.

Disc Diffusion Method

7 mm filter paper discs (Whatman, no. 3) were impregnated with 1ml of *Bacillus* extract. The discs were allowed to remain at room temperature until complete diluent evaporation and were placed onto the surface of the Muller Hinton agar Media agar inoculated with test microorganisms then incubated for 24 hours at 37°C in inverted position. Clear zones of inhibition were developed and were measured (Heatley, 1944).

RESULTS

Collection and Isolation

Total plate counts of bacteria isolated from rhizospheric soil were recorded after 48 hours incubation. Table 1. Showed that maximum bacterial population was found in the rhizospheric soil of *Aloe vera* followed by *Mentha* and *Salvia*. Bacterial colonies obtained from *Aloe vera* rhizospheric soil were identified and designated as Sample no.1.

Table 1: Viable Count (cfu/g) from the Rhizosphere Soil

Sr. No.	Soil isolates	Cfu 10^6
1	*Aloe Vera*	8.02 x 10^6
2	*Mint*	7.09 x10^6
3	*Salvia*	6.08 x10^6

A no. of three clinical isolates was obtained on transfer media and designated as sample no. 2, 3, and 4 and were identified individually. Figure 1. Showed all the bacterial isolates obtained on respective medium.

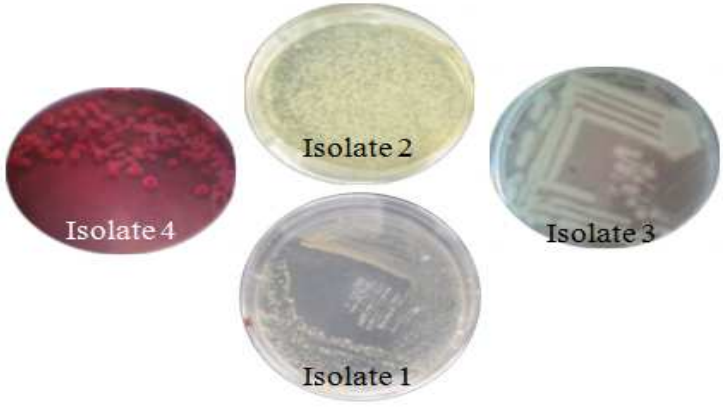

Figure 1: Bacterial Isolate 1 Isolated from *Aloe vera* Rhizosphere and Isolate 2, 3 and 4 Clinically Isolated Bacterial Isolates

Identification of Rhizospheric and Clinical Bacterial Samples

Both morphological and biochemical examination was performed for sample identification.

Table 2: Physiological Characteristics of Selected Isolates with Respective Staining Techniques

Sr. No.	Sample	Gram Staining	Media	Colony Morphology
1.	Sample No. 1	Gram positive rods	Nutrient agar	White, dry, flat, irregular and circular colonies with green coloured endospore and purple coloured capsule.
2.	Sample No.2	Gram negative bacilli	Nutrient agar	Colonies are grey, smooth, translucent, irregularly around and emit a characteristics fruity odour.
3.	Sample No.3	Gram positive cocci	Nutrient agar	White, smooth, convex with entire and shiny margins
4.	Sample No.4	Gram negative cocci	MacConkey agar	Colonies are bright pink circular and convex.

Biochemical characterization of the isolates was done using various biochemical media. All isolates grew on a variety of sugars and isolates showing difference in biochemical treatments as shown in Figure 2.

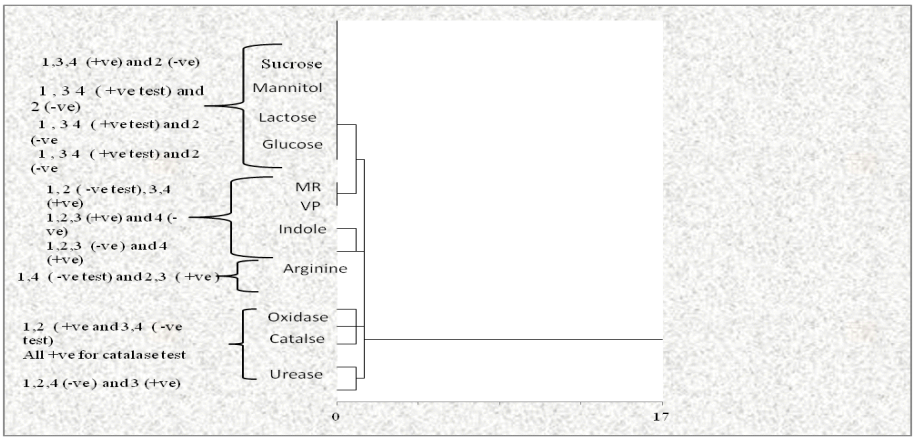

Figure 2: Dendrogram Showing Biochemical Test of Isolates Based on Rescaled Distance

ABIS identification based on morphological and biochemical tests revealed isolate 1 as *Bacillus* and isolates 2, 3 and 4 as *Pseudomonas*, *Staphylococcus* and *E.coli* respectively.

Antibiotic Sensitivity Test

Agar well Diffusion Method

During agar well diffusion method an inhibition zone of 26mm was observed as highest when supernatant from *Bacillus* was screened against *Pseudomonas* followed by *Staphylococcus* (24mm) and *E.coli* (22mm), respectively (Figure 3 and 5).

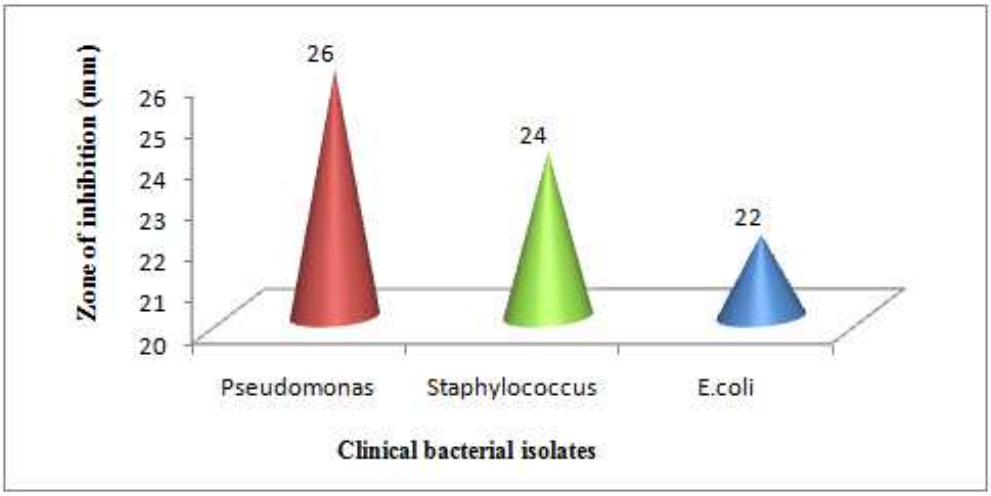

Figure 3 Zone of Inhibition (mm) of Clinical Isolates

Disc Diffusion Method

During disc diffusion method an inhibition zone of 28 mm was observed as highest when supernatant was screened against *Pseudomonas* followed by *Staphylococcus* (26mm) and *E.coli* (24mm), respectively (Figure 4).

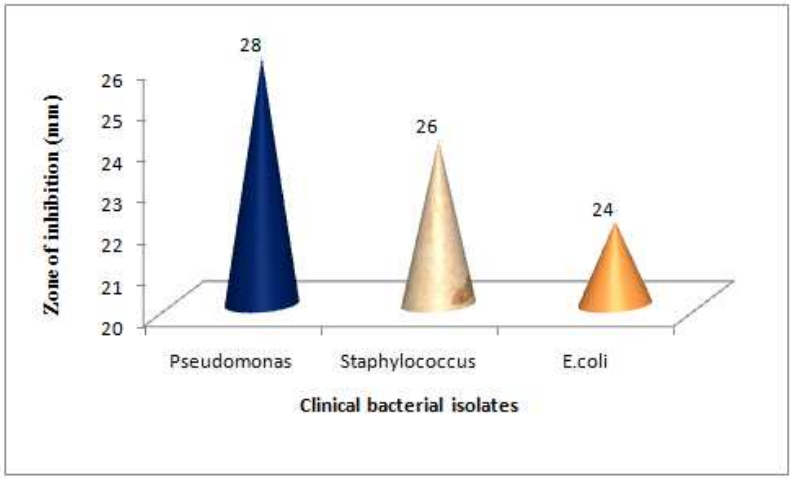

Figure 4: Zone of Inhibition (mm) of Clinical Isolates

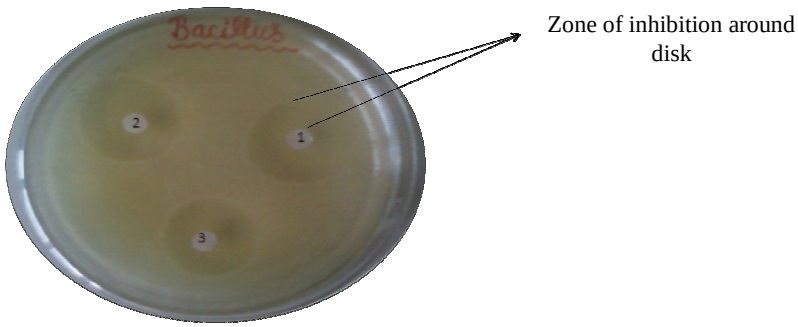

Figure 5: *Bacillus* Extract Disk with Lawn Culture of Pathogenic Isolates

DISCUSSIONS

We explore rhizosphere as it has very dynamic environment and harbors a variety of microorganisms. In the present investigation soil samples were collected from the rhizosphere of medicinal plants from botanical garden of Abhilashi University (H.P.). Soil samples (3 in no.) were collected and isolated on nutrient media as well as on selective media. Phenotypic characterization was done by colony morphology and biochemical testing. We isolate *Bacillu*s from rhizospheric part of *Aloe vera*, *Mentha* and *Salvia*. Lawrence et al., (2009) isolated, purified and evaluated antibacterial agents from *Aloe vera*. Arora *et al*., (2013) isolated and characterized antibiotic producing microbes present in rhizospheric soil. Organism was preferred for isolation as it one of the useful microorganism produce a large number of antibiotics (Tamilarasi et al., 2008; Pandey and Malviya, 2014). Sensitivity test was done on Muller Hinton agar Media (Kirby et al., 1966) and then effect of antibiotic produced by microorganism was examined against pathogenic microorganisms including *S. aureus, E. coli* and *Pseudomonas*. It was concluded that *Bacillus* effects the growth of microbes and formed a zone of inhibition by both Agar Well diffusion method as well as Disc diffusion method. Antibiotics are affective only against bacteria because they attack the unique peptidoglycan cell wall or smaller ribosomal unit of the bacteria (Butler and AD Buss, 1944). Thus, in present paper the organism was tested against *E. coli*, *Staphylococcus*, *Pseudomonas* spp. Results of antibiotics activity expressed in terms of the diameter of the inhibition zone. Maximum inhibition caused by *Bacillus* producing was screened against *Pseudomonas* (28mm) followed by *Staphylococcus* (26mm) and *E.coli* (24mm), respectively in disc diffusion method.

CONCLUSIONS

From the extensive literature review, it is evident that soil is an important part of the earth and can also serve as a good medium for growth of microorganisms. These microorganisms have the property of producing antibiotics and therefore useful from medicinal point of view. The pathogenicity of undesired microorganisms and medicinal property of useful microorganisms were reviewed in this study and hence one of the aims of this study was to assess the antibiotic producing microorganisms. There is often one source of bacteria such as *Bacillus* which showed a strong antimicrobial activity against the pathogenic microorganisms. *Bacillus* isolated from soil has antimicrobial activities, hence used as an antimicrobial agent against isolated pathogenic microbes.

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